International Journal of Biology; Vol. 8, No. 2; 2016
ISSN 1916-9671 E-ISSN 1916-968X
Published by Canadian Center of Science and Education

Stochastic Food Deprivation has no Effect on Cellular Immunity in the Striped Field Mouse (*Apodemus agrarius*)

Xu De-Li[1]*, Bi Shi-Jia[1]*, Hu Xiao-Kai[1], Zhang Kai-Yue[1], Lü Ying-Jie[1], Li Dan[1], Wang Liu-Hong[1], Yao Cong-Min[1], Wang Shan-Shan[1] & Guo Qi[1]

[1] College of Life Sciences, Qufu Normal University, Qufu, 273165, Shandong Province, China

Correspondence: Xu De-Li, College of Life Sciences, Qufu Normal University, Qufu, 273165, Shandong Province, China. Tel: 86-537-4458-281. E-mail: xudl1975@163.com

* These two authors contributed equally to this work

Received: December 9, 2015 Accepted: December 16, 2015 Online Published: March 5, 2016

doi:10.5539/ijb.v8n2p58 URL: http://dx.doi.org/10.5539/ijb.v8n2p58

Abstract

Immune system protects animals from the attack of pathogens, which is crucial to their survival and fitness. Small mammals in the temperate area are confronted with unpredictable food shortage frequently due to the fluctuation of food resources seasonally. Our previous research has shown that stochastic food deprivation (FD) increases T cell-mediated immunity in Kunming mice. In the present study, we tested the hypothesis that stochastic food deprivation would also increase T cell-mediated immunity in the striped field mouse. Nine female striped field mouse were randomly divided into the Fed (n = 5) and FD (n = 4) groups, in which the latter were subjected to stochastic FD regime. Contrary to our expectation, stochastic food deprivation had no significant effect on phytohaemagglutinin (PHA) response indicative of T cell-mediated immunity in striped field mouse compared with the fed controls. Moreover, body mass, body fat mass, wet thymus and spleen mass, white blood cells and blood glucose levels did not differ between the Fed and FD groups. Taken together, the striped field mouse could maintain stable cellular immunity in face of unpredictable food shortage.

Keywords: *Apodemus agrarius*, phytohaemagglutinin response, stochastic food deprivation

1. Introduction

Immune function, which protects animals from the attack of pathogens, is an important factor to determine their survival and fitness (Sheldon & Verhulst, 1996; Owens & Wilson, 1999). Food resources fluctuate seasonally and hence unpredictable food shortage is common to small mammals in the temperate area (Nelson & Demas, 1996; Martin et al., 2008). Food shortage has great impact on animals' immunity (Calder & Kew, 2002; Kaminogawa & Nanno, 2004; Schaible et al., 2007).

Some researchers found that acute food deprivation had suppressive effect on immune function. For instance, two-day food deprivation inhibited T-cell immune response (Lord et al., 1998) and increased susceptibility to endotoxic shock in mice (Faggioni et al., 2000). Delayed type hypersensitivity (DTH) indicative of T-cell immune response were also suppressed in starved rats or mice in contrast with the fed animals (Nohr et al., 1985; Nakamura et al., 2001, 2004). Similarly, DTH was decreased after 7-day intermittent starvation in mice (Kohno et al., 2011). Our previous research has shown that T-cell mediated immunity was suppressed after 3-day food deprivation in wild Mongolian gerbils (*Meriones unguiculatus*) (Xu & Wang, 2010; Xu & Wang, 2015). However, other researchers obtained disparate findings. Two or three days of food deprivation increased the resistance to the intracellular pathogen *Listeria monocytogenes* in mice (Wing & Young, 1980). Moreover, the phagocytic activity of macrophages (Kubo et al., 1982) and blood monocyte bactericidal activity (Wing et al., 1983) in mice increased after a short-term of starvation. In addition, the skin wound healing indicative of innate immunity was accelerated after repeatedly short-term starvation in mice (Hayati et al., 2011). We have found that stochastic food deprivation could increase cellular immunity in Kunming mice (Xu et al., 2014). Therefore, more researches are needed to clarify these different results in more species.

Phytohaemagglutinin (PHA) response involves a subcutaneous injection of PHA that induces local T-cell stimulation and proliferation resulting in swelling (Smits et al., 1999). It has been used to assessed mammalian T cell immunity (Webb et al., 2003; Bellocq et al., 2006). Moreover, immune organs including thymus are indirect

immunological indices (Savino & Dardenne, 2000; Calder & Kew, 2002; Smith & Hunt, 2004). Total white blood cells (WBC) are also used to evaluated the overall health (Calder & Kew, 2002). Adipose tissues are no longer considered as simply passive energy depots, but have been recently regarded as important endocrine and immune organs (Ahima & Flie, 2000; Trayhurn, 2005; Fantuzzi, 2005; Schäffler et al., 2007).

Striped field mice (*Apodemus agrarius*) live widely in China (Zhang & Wang, 1998). Grassland, farmland, old cultivated field and hillsides are their primary habitats (Ma, 1986). Researches have been carried out on the population dynamics (Zhu & Qin, 1991), physiological and behavioral traits (Liu et al., 2006; Yan et al., 2010) in striped field mice. However, we still don't know how immune function responds to unpredictable food shortage in striped field mice. Based on our previous findings that stochastic food deprivation enhanced T cell-mediated immune response in Kunming mice (Xu et al., 2014), we expected that stochastic food deprivation would also increase T cell-mediated immune response in striped field mice.

2. Materials and Methods

2.1 Animals and experimental design

All animal procedures were licensed under the Institutional Animal Care and Use Committee of Qufu Normal University. Striped field mice were wild captured in Juye town of Shandong province (E116°5′N35°24′) during April 10th to May 20th in 2014. Then they were carried to the animal feeding room of Qufu Normal University. Striped field mice were housed individually in plastic cages (30cm×15cm×20cm) with sawdust as bedding under a constant photoperiod of 12L:12D (12h:12h light-dark cycle) and temperature of 23±2℃. Standard rat pellets chow (Animal Breeding Center in Jining Medical College, Jining, China) and water were provided *ad libitum*. After body mass stabilized, 11 female mice were randomly divided into the fed *ad libitum* (Fed) group (n=5) and stochastic food deprivation (FD) group (n=6). Day 0 and day n represented initial day and n days of treatment, respectively. The time of food deprivation was on day 1, 2, 5, 8, 10, 13, 16, 18, 21. The experimental time lasted for 22 days in the present study. One striped field mouse in the FD group died on day 4 and another one in the Fed group died on day 16. The data of these two mice were not included in the subsequent statistical analysis.

2.2 Cellular Immunity Assays

PHA response indicative of cellular immune response was assessed as described previously (Bellocq et al., 2006; Xu & Wang, 2010). Specifically, striped field mice in the Fed and FD groups on day 19 were caught, then we measured their footpad thickness of the left hind foot with a micrometer (Digimatic Indicator ID-C Mitutoyo Absolute cod. 547-301, Japan) to ± 0.01 mm. Immediately thereafter, mice in the Fed and FD groups were injected subcutaneously 0.1 mg of PHA (PHA-P, Sigma L-8754) dissolved in 0.03 mL of sterile saline (pH7.4） in the middle of the footpad. After 6 h, 12h, 24 h, 48 h and 72 h injection, we measured footpad thickness. The PHA response (i.e., cellular immunity) was calculated as the difference between pre- and post- injection measurements divided by initial footpad thickness (PHA response = (post PHA – pre PHA) / pre PHA). Six measures of footpad thickness were taken to obtain the value of each animal (Xu & Wang, 2010).

2.3 Body Composition

Body composition was examined as described previously (Xu & Wang, 2010). In brief, the visceral organs, including heart, thymus, lungs, liver, spleen, kidneys, adrenal glands, gonads (ovaries and uterus) and the digestive organs with contents (i.e., stomach, small intestine, caecum and colon) were dissected and weighed (± 1mg). The stomach, small intestine, caecum and colon were rinsed with saline to eliminate all the gut contents, before being weighed. Moreover, subcutaneous fat, retroperitoneal fat and mesenteric fat were also dissected carefully and weighted. All the three parts of fat mass was regarded as total body fat mass. Subcutaneous fat content, retroperitoneal fat content, mesenteric fat content and total body fat content were calculated as their respective fat mass divided by final body mass.

2.4 White Blood Cells Assays

At the end of the experiment, after collecting trunk blood, 20 μL whole blood was diluted immediately in 0.38 mL solution containing 1.5% glacial acetic acid, 1% crystal violet (Sigma) and the leukocytes were counted in an improved Neubauer chamber using microscope. The total number of WBC was determined by counting all leucocytes in the four corner large-squares of the Neubauer chamber, and multiplying the raw data by 5×10^7 to obtain the final values (10^9cells/L) (Yang, 2004).

2.5 Blood Glucose Assays

Blood glucose levels were measured with FreeStyle Mini Blood Meter (Abbott Diabetes Care Inc. Alameda, USA) according to the manufacture's instructions. The range tested of blood glucose was 1.1-27.8mmol/L. The within-lot and -vial precision are <5.6% and <4.1%, respectively

3. Statistical Analysis

Data were analyzed using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). Prior to all statistical analyses, data were examined for normality and homogeneity of variance, using Kolmogorov–Smirnov and Levene tests, respectively. The differences of body mass between the Fed and FD groups were analyzed by independent-samples t-test. Group differences in wet organ mass with body mass as the covariate were analyzed by General Linear Model multivariate analysis followed by Bonferroni *post hoc* tests. Group differences in other parameters (body compositions, PHA response, WBC, blood glucose levels concentrations) were analyzed by independent-samples t-test. Pearson correlation analysis was performed to determine the correlations of PHA response with blood glucose and body fat mass in the Fed and FD groups. Results were expressed as mean ± SE, and $P<0.05$ was considered to be statistically significant.

4. Results

4.1 Body Mass

On day 8 ($t = -2.668$, $df = 7$, $P = 0.032$), day 9 ($t = -2.925$, $df = 7$, $P = 0.022$), day 10 ($t = -2.617$, $df = 7$, $P = 0.035$), day 11 ($t = -3.822$, $df = 7$, $P = 0.007$), day 14 ($t = -2.401$, $df = 7$, $P = 0.047$), day 15 ($t = -2.401$, $df = 7$, $P = 0.047$), day 18 ($t = -2.991$, $df = 7$, $P = 0.020$), the percent of body mass of initial day decreased in the FD group compared to the Fed group (Figure 1). There was no significant difference of the percent of body mass on initial day between the two groups on other days (Figure 1).

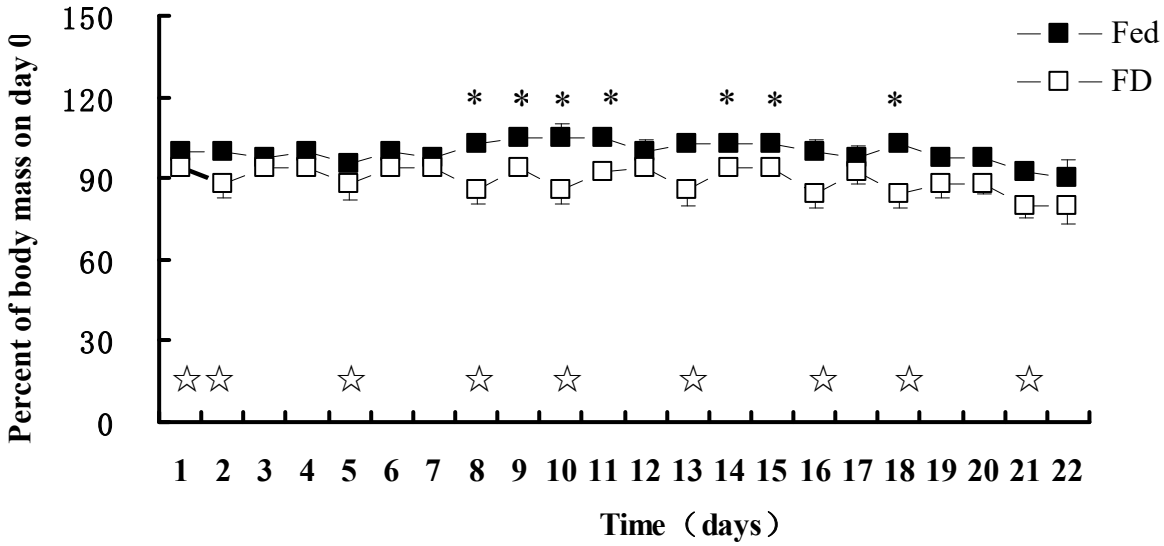

Figure 1. Changes of the percent of body mass on initial day in field mice during stochastic food deprivation. Values are means ± SE. Note: ■=Fed group, □= FD group. An asterisk (*) indicates statistical differences at P＜ 0.05, and the symbol of "☆"indicates the day of food deprivation

4.2 Body Composition

Stochastic food deprivation significantly increased stomach wet mass in striped field mice in contrast with the fed controls (Table 2), whereas there were no differences of body composition and other wet organ masses between the Fed and FD groups (Table 1, 2).

Table 1. Effect of stochastic food deprivation on body composition in striped field mice.

Parameters	Fed group	FD group	Statistical summary	
Sample size	4	5	$F_{1,7}$	*P*
Final body mass (g)	19.8±2.5	20.2±2.3	0.016	0.902
Wet carcass mass (g)	15.8±2.5	14.0±2.2	0.286	0.609
Subcutaneus fat (g)	0.467±0.223	0.128±0.199	1.286	0.294
Subcutaneus fat content (%)	2.30±0.9	0.80±0.08	1.737	0.229
Retroperitoneal fat (g)	0.038±0.025	0.019±0.022	0.344	0.576
Retroperitoneal fat content (%)	0.20±0.01	0.10±0.009	0.160	0.701
Mesenteric fat (g)	0.279±0.096	0.153±0.086	0.946	0.363
Mesenteric fat content (%)	1.60±0.4	1.00±0.4	0.920	0.369
Total body fat (g)	0.785±0.327	0.301±0.293	1.216	0.307
Total body fat content (%)	4.10±0.13	1.90±0.11	1.615	0.244

Values are means ± SE. Values for a specific parameter that share different superscripts are significantly different at P<0.05, determined by independent-samples t-test.

Table 2. Effect of stochastic food deprivation on wet organ mass in striped field mice.

Parameters	Fed group	FD group	Statistical summary	
Sample size	4	5	$F_{1,6}$	*P*
BAT (g)	0.249±0.080	0.102±0.072	1.853	0.222
Brain (g)	0.407±0.015	0.380±0.014	1.790	0.229
Heart (g)	0.139±0.007	0.135±0.006	0.235	0.645
Lungs (g)	0.176±0.027	0.178±0.024	0.005	0.947
Thymus (g)	0.006±0.002	0.005±0.001	0.229	0.649
Liver (g)	0.919±0.077	0.800±0.069	1.339	0.291
Spleen (g)	0.029±0.005	0.032±0.005	0.175	0.690
Kidneys (g)	0.290±0.005	0.263±0.015	1.515	0.264
Adrenal glands (g)	0.007±0.000	0.006±0.000	3.283	0.120
Gonads	0.049±0.010	0.019±0.009	5.150	0.064
Stomach with contents (g)	0.882±0.153	0.457±0.137	4.279	0.084
Stomach (g)	0.177±0.014	0.227±0.013	7.013	0.038
Small intestine with contents (g)	1.040±0.194	0.846±0.173	0.559	0.483
Small intestine (g)	0.367±0.036	0.443±0.032	2.380	0.174
Small intestine length (cm)	31.578±1.629	30.837±1.457	0.115	0.746
Caecum with contents (g)	0.541±0.104	0.667±0.093	0.812	0.402
Caecum (g)	0.171±0.028	0.195±0.025	0.409	0.546
Caecum length (cm)	5.581±0.813	5.537±0.727	0.036	0.856
Colon with contents (g)	0.320±0.061	0.296±0.054	0.086	0.779
Colon (g)	0.146±0.019	0.119±0.017	1.138	0.327
Colon length (cm)	9.861±0.439	8.612±0.393	4.488	0.078
Total digestive tract (g)	0.685±0.054	0.757±0.048	0.991	0.358
Total digestive tract length (cm)	47.020±2.523	44.824±2.256	0.421	0.541

Values are means ± SE. Values for a specific parameter that share different superscripts are significantly different at P<0.05, determined by General Linear Model multivariate analysis followed by Bonferroni *post hoc* tests with body mass as the covariate ns, not significant.

4.3 White Blood Cells

Stochastic food deprivation had no significant effect on WBC in striped field mice (t=-1.703, df=7, P=0.132) (Figure 2).

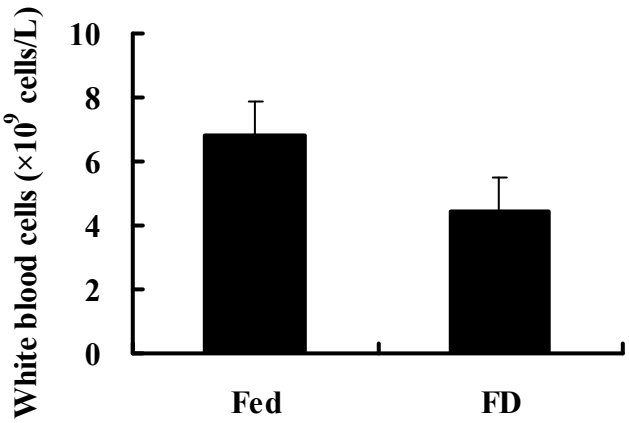

Figure 2. Effect of stochastic food deprivation on white blood cells in striped field mice. Values are means ± SE. WBC did not differ between the Fed and FD groups. The solid column represents the Fed group and white column stands for the FD group

4.4 Cellular Immune Response

There was on significant difference of PHA response between the Fed and FD groups after 6h (t=1.150, df=7, *P*=0.288), 12h (t=0.314, df=7, *P*=0.763), 24h (t=-0.957, df=7, *P*=0.370), 48h (t=0.238, df=7, *P*=0.819), 72h (t=-0.183, df=7, *P*=0.860) of PHA postimmunization (Figure 3). Total body fat mass was not correlated with PHA response after 6h (r=0.255, *P*=0.508), 12h (r=-0.094, *P*=0.810), 24h (r=-0.202, *P*=0.603), 48h (r=-0.279, *P*=0.467), 72h (r=0.066, *P*=0.865) of PHA injection in the Fed and FD groups.

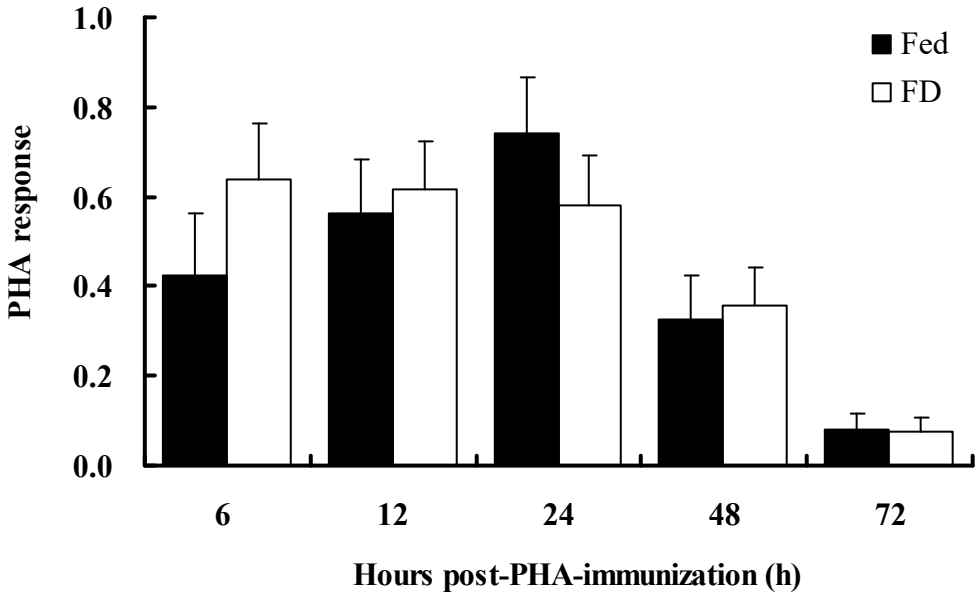

Figure 3. Effect of stochastic food deprivation on PHA response in striped field mice. Values are means ± SE. The solid column represents the Fed group and white column stands for the FD group

4.5 Blood Glucose

Blood glucose level did not differ between the FD and Fed groups (t=-0.898, df=7, *P*=0.399) (Figure 4). It was not correlated with PHA response after 6h (r=0.072, *P*=0.853), 12h (r=-0.097, *P*=0.805), 24h (r=-0.200, *P*=0.605), 48h (r=-0.322, *P*=0.398), 72h (r=-0.232, *P*=0.547) of PHA injection in the Fed and FD groups. However, blood glucose level was positively correlated with total body fat mass (r=0.916, *P*=0.001).

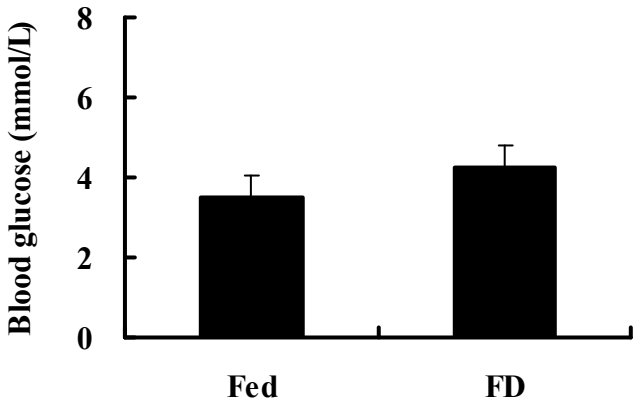

Figure 4. Effect of stochastic food deprivation on blood glucose level in mice. Values are means ± SE. Blood glucose level was not different between the Fed and FD groups

5. Discussion

Contrary to our prediction, T cell-mediated immunity was not influenced by stochastic FD in striped field mice. Immune organs and WBC were also not affected by stochastic FD.

Adipose tissues are important energy reserves which provide energy for many physiological processes such as immune responses (Trayhurn, 2005; Steiner & Romanovsky, 2007). Maintaining and mounting immune responses are costly in term of energy (Demas et al., 1997; Moret & Schmid-Hempel, 2000). Generally, animals with low energy reserves would choose to allocate less energy to immune defense than animals with higher reserves (Houston et al., 2007). In the present study, there was no significant differences of the masses of subcutaneus fat, retroperitoneal fat, mesenteric fat and total body fat between the Fed and FD striped mice. Additionally, total body fat was not correlated with PHA response. These results disagreed with our previous finding in which stochastic FD increased T cell-mediated immunity in Kunming mice (Xu et al., 2014). The differences of species used and the experimental regime might account for the discrepancies.

Glucose as a metabolic fuel is required for normal survival and function of lymphocytes, and its metabolism plays an important role in T-cell activation and proliferation (Maciver et al., 2008; Xu & Wang, 2011). Consequently, glucose uptake and glycolysis increase during an immune response (Matarese & Cava, 2004). In the present study, blood glucose level in the FD striped field mice was similar with that of the fed controls, which indicated that the FD striped field mice were not short of metabolic fuels. Taken together, glucose provided important energy for costly biological processes including T cell-mediated immune response (Sheldon et al., 1996; Moret et al., 2000).

In summary, stochastic food deprivation had no effect on cellular immunity, immune organs (i.e., thymus and spleen) and white blood cells in the FD striped field mice. The reasons might be due to the insignificant changes of body fat mass and blood glucose during stochastic FD treatment. Small sample size and the unknown ages of the striped field mice might also account for our results. Further research is needed to investigate whether other immunological parameters such as humoral and innate immunity would be affected by stochastic FD in this species.

Acknowledgements

The present study was supported by grants from the National Natural Science Foundation of China (No.31370427), Natural Science Foundation of Shandong province (ZR2013CM019) to DLX.

References

Ahima, R. S., & Flier, J. S. (2000). Adipose tissue as an endocrine organ. *Trends Endocrinol. Metab., 11*, 327-332.

Bellocq, J. G., Krasnov, B. R., Khokhlova, I. S., & Pinshow, B. (2006). Temporal dynamics of a T-cell mediated immune response in desert rodents. *Comp. Biochem. Physiol. A, 145*, 554-559.

Calder, P. C., & Kew, S. (2002). The immune system: a target for functional foods? *Bri. J. Nutr., 88*, S165-176.

Demas, G. E., Chefer, V., Talan, M. I., & Nelson, R. J. (1997). Metabolic costs of mounting an antigen-stimulated immune response in adult and aged C57BL/6J mice. *Am. J. Physiol. Reg. Integr. Comp. Physiol., 273*, 1631-1637.

Faggioni, R., Moser, A., Feingold, K. R., & Grunfeld, C. (2000). Reduced leptin levels in starvation increase susceptibility to endotoxic shock. *Am. J. Pathol., 156*, 1781-1787.

Fantuzzi, G. (2005). Adipose tissue, adipokines, and inflammation. *J. Allergy Clin. Immunol., 115*, 911-919.

Hayati, F., Maleki, M., Pourmohammad, M., Sardari, K., Mohri, M., & Afkhami, A. (2011). Influence of short-term, repeated fasting on the skin wound healing of female mice. *Wounds, 23*, 38-43.

Houston, A. I., McNamara, J. M., Barta, Z., & Klasing, K. C. (2007). The effect of energy reserves and food availability on optimal immune defence. *Proc. R. Soc. B, 274*, 2835-2842.

Kaminogawa, S., & Nanno, M. (2004). Modulation of immune functions by foods. *eCAM., 1*, 241-250.

Kohno, H., Kouda, K., Ishihara, H., Nishio, N., Sasaki, Y., Nakamura, H., Iki, M., & Sonoda, Y. (2011). Intermittent fasting ameliorates delayed-type hypersensitivity in NC/Nga mice. *Food and Nutrition Sciences, 2*, 287-293.

Kubo, C., Teshima, H., & Ago, Y. (1982). The effects of acute starvation on the function of immune system. *Jpn J Psychosom Med., 22*, 249-254.

Liu, J. S., Sun, R. Y., & Wang, D. H. (2006). Thermogenic properties in three rodent species from Northeastern China in summer. *J Therm Biol, 31*, 172-176.

Lord, G. M., Matarese, G., Howard, J. K., Baker, R. J., Bloom, S. R., & Lechler, R. I. (1998). Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. *Nature, 394*, 897-901.

Ma, Y. Q. (1986). *Mammals of Heilongjiang Province* (pp. 305-376). Science and Technology Press, Heilongjian.

Maciver, N. J., Jacobs, S. R., Wieman, H. L., Wofford, J. A., Coloff, J. L., & Rathmell, J. C. (2008). Glucose metabolism in lymphocytes is a regulated process with significant effects on immune cell function and survival. *J. Leuko. Biol., 84*, 949-957.

Martin, L. B., Weil, Z. M., Bowers, S. L., & Nelson, R. J. (2008). Sex-specific effects of glucose deprivation on cell-mediated immunity and reproduction in Siberian hamsters (*Phodopus sungorus*). *J Comp. Physiol. B, 178*, 623-628.

Matarese, G., & Cava, A. L. (2004). The intricate interface between immune system and metabolism. *Trends Immunol., 25*, 193-200.

Moret, Y., & Schmid-Hempel, P. (2000). Survival for immunity: the price of immune system activation for bumblebee workers. *Science, 290*, 1166-1168.

Nakamura, H., Koud, K., Tokunaga, R., & Takeuchi, H. (2004). Suppressive effects on delayed type hypersensitivity by fasting and dietary restriction in ICR mice. *Toxicol. Lett., 146*, 259-267.

Nakamura, H., Kouda, K., Fan, W. Y., Watanabe T., & Takeuchi, H. (2001). Suppressive effects on allergic contact dermatitis by short-term fasting. *Toxicol. Pathol., 29*, 200-207.

Nelson, R. J., & Demas, G. E. (1996). Seasonal changes in immune function. *Q Rev Biol, 71*, 512-548.

Nohr, C. W., Tchervenkov, J. I., Meakins, J. L., & Christou, N. V. (1985). Malnutrition and humoral immunity: short term acute nutritional deprivation. S*urgery, 98*, 769-775.

Owens, I. P. F., & Wilson, K. (1999). Immunocompetence: a neglected life history trait or conspicuous red herring? *Trends Ecol. Evol., 14*, 170-172.

Savino, W., & Dardenne, M. (2000). Neuroendocrine control of thymus physiology. *Endocr. Rev., 21*, 412-443.

Schäffler, A., Schölmerich, J., & Salzberger, B. (2007). Adipose tissue as an immunological organ: Toll-like receptors, C1q/TNFs and CTRPs. *Trends Immunol., 28*, 393-399.

Schaible, U. E., & Kaufmann, S. H. E. (2007). Malnutrition and infection: complex mechanisms and global impacts. *Plos Med., 4*, 806-812.

Sheldon, B. C., & Verhulst, S. (1996). Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends Ecol. Evol., 11*, 317-321.

Smith, K. G., & Hunt, J. L. (2004). On the use of spleen mass as a measure of avian immune system strength. *Oecologia, 138*, 28-31.

Smits, J. E., Bortolotti, G. R., & Tella, J. L. (1999). Simplifying the phytohaemagglutinin skin-testing technique in studies of avian immunocompetence. *Funct. Ecol., 13*, 567–572.

Steiner, A. A., & Romanovsky, A. A. (2007). Leptin: at the crossroads of energy balance and systemic inflammation. *Prog. Lipid Res., 46*, 89-107.

Trayhurn, P. (2005). Endocrine and signalling role of adipose tissue: new perspectives on fat. *Acta Physiol. Scand., 184*, 285-293.

Wing, E. D., Stanko, R. T., Winkelstein, A., & Adibi, S. A. (1983). Fasting enhanced immune effector mechanisms in obese subjects. *Am J Med., 75,* 91–96.

Wing, E. J., & Young, J. B. (1980). Acute starvation protects mice against *Listeria monocytogenes*. *Infect. Immun., 28*, 771-776.

Xu, D. L., Tian, Y. F., Li, R. S., Li, H. T., Sun, X. N., Wang, Q. B., Shi, J., Zhou, P., & Sun, X. L. (2014). Stochastic food deprivation increases cellular immunity in Kunming Mice. *Health, 6*, 1099-1107.

Xu, D. L., & Wang, D. H. (2010). Fasting suppresses T cell-mediated immunity in female Mongolian gerbils (*Meriones unguiculatus*). *Comp Biochem Physiol A, 155*, 25-33.

Xu, D. L., & Wang, D. H. (2011). Glucose supplement reverses the fasting-induced suppression of cellular immunity in Mongolian gerbils (*Meriones unguiculatus*). *Zoology, 114*(5), 306-312.

Xu, D. L., & Wang, D. H. (2015). Effect of fasting duration on immune function in female Mongolian gerbils (*Meriones unguiculatus*). *Acta Theriologica Sinica., 35*(4), 369-378.

Yan, B., Jin, Z. M., Yang, C. W., Liu, Z., & Li, D. W. (2010). Study on the hoarding behavior of *Apodemus agrarius*. *Journal of Anhui Agri Sci., 38*(5), 2389-2390.

Yang, X. P. (2004). *Laboratory manual in animal physiology* (1st ed. Pp. 91-94). Higher Education Press.

Zhang, Z. B., & Wang, Z. W. (1998). *Ecology and Management of Rodent Pests in Agriculture*. Ocean Publishing House, Beijing.

Zhu, S. K., & Qin, Z. H. (1991). Analysis of population dynamics of *Apodemus agrarius* in Huaibei area of Anhui province. *Acta Theriologica Sinica., 11*, 213-219.